TARGETED HPLC-UV-FLD POLYPHENOLICS TO ASSESS PAPRIKA GEOGRAPHICAL ORIGIN

Xavi Collado [1], Guillem Campmajó [1,2], Sònia Sentellas [1,3], Javier Saurina [1,2], Oscar Núñez [1,2]

(1) Department of Chemical Engineering and Analytical Chemistry, University of Barcelona. E08028, Barcelona, Spain.

(2) Research Institute in Food Nutrition and Food Safety, University of Barcelona. E08921, Santa Coloma de Gramenet, Spain.

(3) Serra Húnter Lecturer, Generalitat de Catalunya. E08007, Barcelona, Spain.

E-mail: oscar.nunez@ub.edu

1. INTRODUCTION

Paprika is a red powder seasoning with a characteristic flavour obtained from the drying and grinding of red pepper fruits of the genus Capsicum (Solanaceae family). In Europe, seven paprika products are distinguished with the protected designation of origin (PDO) label, which ensures a high-quality product by strict requirements, leading to higher retail prices than not-labelled paprika and making them susceptible to fraudulent practices.

Contents of polyphenol and phenolic compounds depend on several factors, such as the environmental conditions of the production area. Thus, in the present study, a simple and feasible high-performance liquid chromatography with ultraviolet and fluorescent detection (HPLC-UV-FLD) method was developed to determine 17 polyphenols in paprika samples, aiming at their authentication through chemometrics.

2. TARGETED POLYPHENOLS

Phenolic acids:

- Gallic acid
- Homogentisic acid
- Chlorogenic acid
- Caffeic acid
- Homovanillic acid
- *p*-Coumaric acid
- Ferulic acid
- Sinapic acid
- Rosmarinic acid

Phenolic aldehydes:

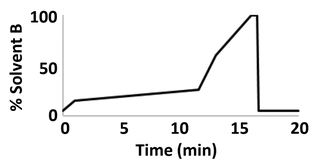
- Protocatechuic aldehyde
- Syringaldehyde

Flavonoides:

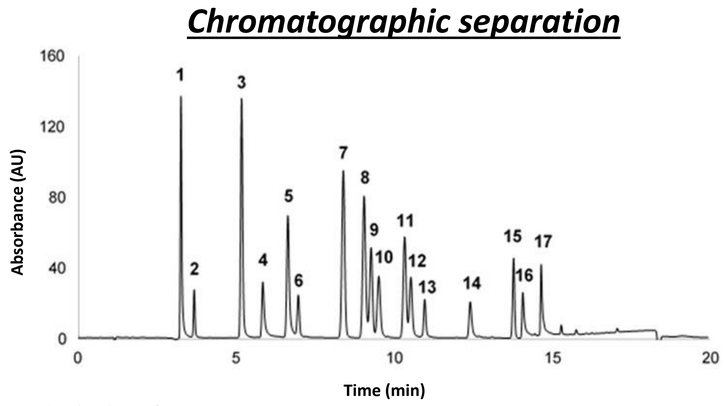
- Umbelliferon
- Rutin
- Nepetin-7-glucoside
- Quercetin

3. HPLC-UV-FLD METHOD

Experimental conditions

Instrument	- Agilent 1100 Series HPLC instrument
Column	- Kinetex® core-shell C18 reversed-phase column (Phenomenex) (100 × 4.6 mm i.d., 2.6 µm particle size) Precolumn: C18 2 mm x 4.6 mm, 2.6 µm
Flow-rate	- 1.0 mL /min
Injection volume	- 10 µL
Gradient elution	- Solvent A: 0.1% formic acid in water (*v/v*) - Solvent B: Acetonitrile 
Acquisition	- UV: 230, 250, 280 and 320 nm - FLD: 320 nm (excitation) and 440 nm (emission)

Chromatographic separation



Peak identification:

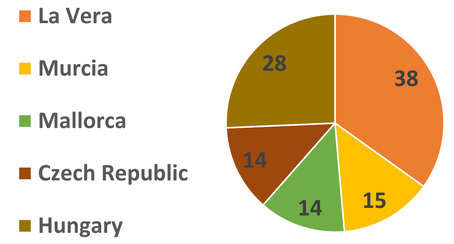
1, gallic acid; 2, homogentisic acid; 3, protocatetic aldehyde; 4, chlorogenic acid; 5, caffeic acid; 6, homovanillic acid; 7, vanillin; 8, p-coumaric acid; 9, syringaldehyde; 10, umbelliferon; 11, ferulic acid; 12, sinapic acid; 13, rutine; 14, nepetin-7-glucoside; 15, hesperidine; 16, rosmarinic acid, and 17, quercetin

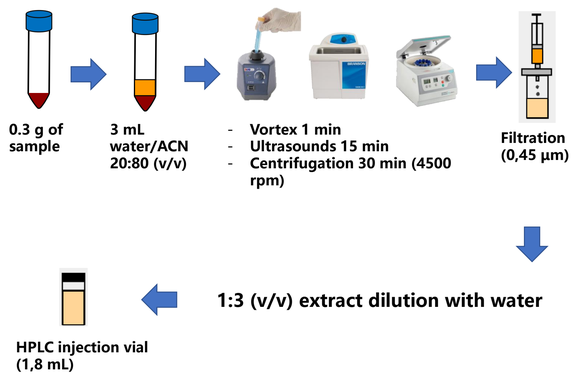
Instrumental Method Performance

LODs (mg/L)	0.004 – 0.87
LOQs (mg/L)	0.01 – 2.9
Linearity (R^2)	0.984 – 0.998
Concentration levels evaluated	0.015, 0.15, 0.75, 3 and 15 mg/L
Precision (% RSD)	Run-to-run precision: 0.1 – 6.2 Day-to-day precision: 0.5 – 23.8
Trueness (% relative error)	0.04 – 8.3

4. SAMPLES AND SAMPLE TREATMENT

109 Paprika samples analyzed

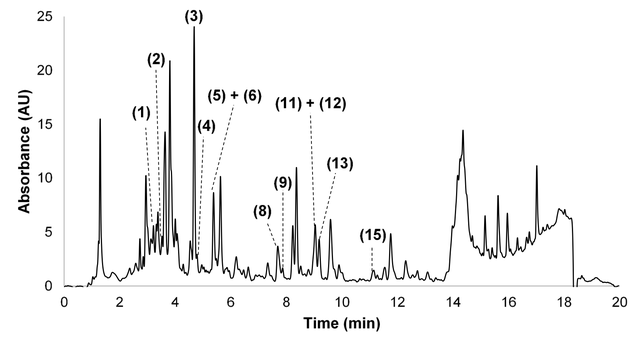




5. Compounds confirmed by HPLC-ESI-MS in paprika

ESI-MS Experimental conditions

MS instrument	400 QTRAP (Ab Sciex)
Ionization source	ESI (-)
Source parameters	Curtain gas: 10 a.u. Auxiliary gas: 50 a.u. Nebulization gas: 50 a.u. ESI voltage: -4.5 kV Temperature: 500 ºC
Acquisition mode	Multiple reaction monitoring (MRM)



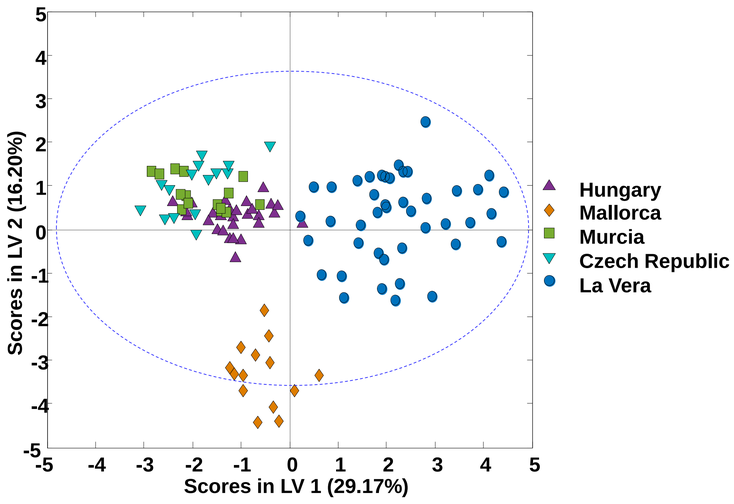
Chromatogram of a Mallorca Paprika sample.

Identified compounds by HPLC-MS:

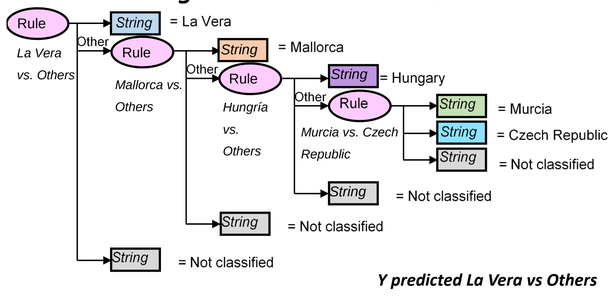
1, gallic acid; 2, homogentisic acid; 3, protocatetic aldehyde; 4, chlorogenic acid; 5, caffeic acid; 6, homovanillic acid; 8, p-coumaric acid; 9, syringaldehyde; 11, ferulic acid; 12, sinapic acid; 13, rutine; 15, hesperidine;

6. CHARACTERIZATION AND CLASSIFICATION OF PAPRIKA SAMPLES BY CHEMOMETRICS

Sample classification by partial least squares-discriminant analysis (PLS-DA)

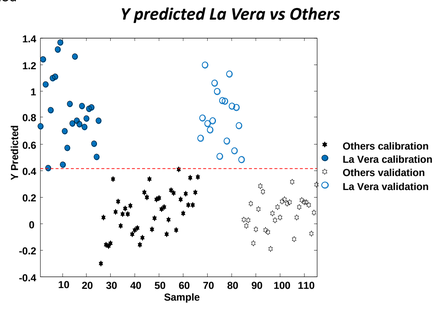


Classification decisión tree by PLS-DA models using Heriarchical Model Builder

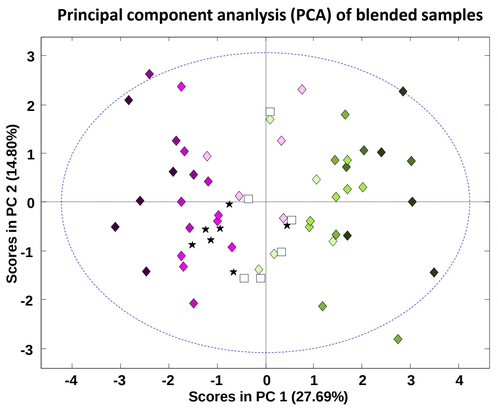


A sample classification rate of 87.8% was reached after external validation

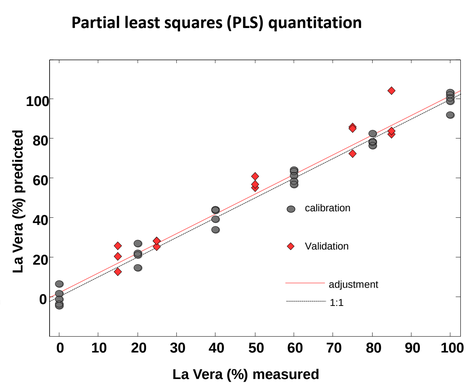
Y predicted La Vera vs Others



Paprika geographicl origin blend evaluation
Scenario: Murcia adulterated with La Vera paprika



Percentage refers to amount of La Vera paprika



PLS allowed blend percentage prediction with errors below 10.8% after external validation

7. SUMMARY AND CONCLUSIONS

- **A HPLC-UV-FLD method has been developed for the determination of 17 polyphenols in paprika samples showing good linearity, LODs, LOQs, precision and trueness.**
- **The presence of the determined polyphenols in paprika samples was confirmed by means of HPLC-ESI-MS/MS using a Q-Trap instrument. A total of 13 polyphenols were identified in the analyzed samples.**
- **Targeted HPLC-UV-FLD polyphenolics showed to be a good methodology to assess paprika geographical origin with a sample classification rate of 87.8% after external validation with PLS-DA. In addition, paprika adulteration percentage prediction with errors bellow 10.8% were achieved.**

8. ACKNOWLEDGEMENTS

The authors thank the financial support received from Agencia Estatal de Investigación (project PID2020-114401RB-C22) and from the Agency for Administration of University and Research Grants (Generalitat de Catalunya) (projects 2017SGR-171 and 2017SGR-310). G. Campmajó thanks to the University of Barcelona for the PhD APIF grant.